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(71) Applicant *(for all designated States except US)*: **HUMAN GENOME SCIENCES, INC.** [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BELL, Adam** [US/US]; 13312 Burnt Woods Place, Germantown, MD 20874 (US). **RUBEN, Steven, M.** [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US).

(74) Agents: **WALES, Michele, M.** et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).

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(54) Title: CHEMOKINE BETA-1 FUSION PROTEINS

(57) Abstract: The present invention relates to novel chemokine polypeptides and encoding nucleic acids. More specifically, therapeutic compositions and methods are provided using isolated nucleic acid molecules encoding a human chemokine beta-1 (Ck$G(b)-1 or Ckb1) polypeptide (previously termed monocyte-colony inhibitory factor (M-CIF), MIP1-gamma, and Hemofiltrate CC chemokine-1 (HCC-1)), and Ckb1 polypeptides themselves, as are vectors, host cells and recombinant methods for producing the same. Also provided are methods of treating, preventing, ameliorating diseases using such compounds.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/16525

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/16, 38/19; C07K 14/00, 14/435, 14/475, 14/52, 19/00
US CL : 530/350; 514/2, 12; 424/85.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/350; 514/2, 12; 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GenBank, EMBL, PIR, SwissProt sequence databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 98/07862 A2 (HUMAN GEOME SCIENCES, INC.) 26 February 1998, see entire document.	1 --- 3,4,6-10,12-14
X --- Y	US 6,180,773 B1 (FORSSMANN et al) 30 January 2001, see entire document.	1 --- 3,4,6-10,12-14
A	SCHULZ-KNAPPE, P. et al. HCC-1, a Novel Chemokine from Human Placenta. Journal of Experimental Medicine. January 1996, Vol. 183, pages 295-299, see entire	1-4, 6-10, 12-14
A	PARDIGOL, A. et al. HCC-2, a human chemokine: Gene structure, expression pattern, and biological activity. Proc. Natl. Acad. Sci. USA. May 1998, Vol. 95, pages 6308-6313, see entire document.	1-4, 6-10, 12-14
A	NOMIYAMA, H. et al. Organization of the Chemokine Gene Cluster on Human Chromosome 17q11.2 Containing the Genes for CC Chemokine MPIF-1, HCC-2, HCC-1, LEC, and RANTES. Journal of Interferon and Cytokine Research. 1999, Vol. 19, pages 227-234, see entire document.	1-4, 6-10, 12-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Yvonne L. Eyler, Ph.D.

Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/16525

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 5
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
the claims recites SEQ ID NO: X. Since no numerical value was provided for the sequence identifier, the sequence search could not be performed.

3. ☒ Claim Nos.: 11, 15-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

least one molecule of a Ckb1 protein (or fragment or variant thereof). Ckb1 protein is also referred to herein as "therapeutic protein". As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Ckb1 protein (or fragment or variant thereof). A fusion protein (e.g. albumin fusion protein) of the invention comprises at least a fragment or variant of a Ckb1 protein and at least a fragment or variant of a heterologous protein (e.g. human serum albumin), which are associated with one another, preferably by genetic fusion (i.e., the fusion protein (e.g. albumin fusion protein) is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Ckb1 protein is joined in-frame with a polynucleotide encoding all or a portion of the heterologous protein (e.g. albumin)) or chemical conjugation to one another. The Ckb1 protein and heterologous (e.g. albumin) protein, once part of the fusion protein, may be referred to as a "portion", "region" or "moiety" of the fusion protein (e.g. albumin fusion protein) (e.g., "Ckb1 protein portion"; "heterologous protein portion"; "albumin protein portion")).

[0055]A fusion protein of the invention comprises, or alternatively consists of, one or more heterologous polypeptides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or more heterologous polypeptides. The heterologous protein may be of any length, from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, etc., amino acids to 100, 500, 1000, etc., amino acids in length. The heterologous proteins may be fused or conjugated anywhere such as at the N-terminus or the C-terminus of Ckb1, and may be of any length. In some preferred embodiments, the heterologous polypeptide is albumin, preferably fused at the C-terminus. In some preferred embodiments, the heterologous polypeptide is a translocation signal, such as a secretion signal, preferably fused at the N-terminus. The translocation signal may be mammalian, vertebrate, eukaryotic, prokaryotic, yeast, bacterial, human, mouse, chicken, E. coli, etc. Preferably, the translocation signal is yeast. In some preferred embodiments, the Ckb1 polypeptide comprises, or alternatively consists of, an N-terminal yeast secretion signal and a C-terminal albumin.

[0056]Human serum albumin (HSA, or HSA), a protein of 585 amino acids in its mature form (as shown in Figure 14 or in SEQ ID NO:5), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HSA for clinical use is produced by extraction from

human blood. The production of recombinant HSA (r HSA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

[0057]The role of albumin as a carrier molecule and its inert nature are desirable properties for use as a carrier and transporter of polypeptides *in vivo*. Fusion of albumin to the Ckb1 protein may be achieved by genetic manipulation, such that the DNA coding for HSA, or a fragment thereof, is joined to the DNA coding for the Ckb1 protein. A suitable host is then transformed or transfected with the fused nucleotide sequences, so arranged on a suitable plasmid as to express a fusion polypeptide. The expression may be effected *in vitro*, for example, prokaryotic or eukaryotic cells, or *in vivo e.g.* from a transgenic organism.

[0058]In one embodiment, the invention provides a fusion protein (e.g. albumin fusion protein) comprising, or alternatively consisting of, a Ckb1 protein (e.g., Ckβ-1) and a serum albumin protein. In other embodiments, the invention provides a fusion protein (e.g. albumin fusion protein) comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Ckb1 protein and a serum albumin protein. In other embodiments, the invention provides a fusion protein (e.g. albumin fusion protein) comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Ckb1 protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the fusion protein (e.g. albumin fusion protein) is the mature portion of serum albumin.

[0059]In further embodiments, the invention provides a fusion protein (e.g. albumin fusion protein) comprising, or alternatively consisting of, a Ckb1 protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides a fusion protein (e.g. albumin fusion protein) comprising, or alternatively consisting of, a Ckb1 protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Ckb1 protein portion of the fusion protein (e.g. albumin fusion protein) is the mature portion of the Ckb1 protein. In a further preferred embodiment, the Ckb1 protein portion of the fusion protein (e.g. albumin fusion protein) is a soluble domain of the Ckb1 protein. In an alternative embodiment, the Ckb1 protein portion of the fusion protein (e.g. albumin fusion protein) is the active form of the Therapeutic protien.

[0060]In a further preferred embodiment, an albumin fusion protein of the invention is processed by a host cell and secreted into the surrounding culture medium, and then

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Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
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gct gca agt caa gct gcc tta ggc ttataacatc tacatttaaa agcatctcag  1782
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Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
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Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
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Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
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Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
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His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
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Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

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